DETAILED ACTION

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 10/25/2010 has been entered.

Claims 1, 3-10, 13-18 and 23-34 are being considered on the merits.

Claim Rejections - 35 USC § 112

1. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

2. Claims 1, 3-9, 23-24, 26-27, and 32 rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

3. Claim 1 is indefinite for "and has no perceivable effect on dough rheology". It is not clear what is meant by 'perceivable'. Should the applicants mean that the action of the added serine protease has no known role or the mechanism is not known, then this limitation does not appear to add anything to claim 1 as far as the patentability of claim 1 is concerned. There is no guidance provided by the specification to show what is meant by 'perceivable'. In other words, the phrase 'no perceivable effect' does not

appear to be further limiting claim 1. The phrase "no effect on dough rheology" is also indefinite. It is not clear what kind of effect the applicants are referring to.

Claim Rejections - 35 USC § 103

4. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

5. Claims 1, 3-4,6, 8-10, 13-15, 17-18, 25, 27, 30-33 are rejected under 35 U.S.C. 103(a) as being unpatentable over Klingenberg et al. (DD 156,714 A; hereinafter R1) in view of Olesen et al. (US 6,110,508; hereinafter R2)

6. R1 teaches preparing a heat stable thermitase from *Thermoactinomyces vulgaris*. This enzyme is a proteinase for weakening gluten in the preparation of wafers, other cereal and bakery products (Page 1, paragraph 1 and Claim 1).

7. R1 teaches of an optimum temperature of thermitase (thermophilic protease) between 60-70C. (page 2, first paragraph).

8. Although there is no explicit disclosure of preventing or retarding staling during the baking process of the bakery products, given that R1 discloses method and improver identical to that presently claimed, it is clear that the method and the improver would intrinsically prevent or retard staling during the baking process of the bakery products.

9. Given that the weakening of gluten is disclosed it is clear that the protease is added to the dough prior to baking as presently claimed.

10. It is also noted that the addition of protease and other enzymes such as amylase in order to increase the shelf life of bread (retard staling) is an old and known process in the art. Patents US 6,270,813 discloses the use of amylase and protease in retarding staling in bread; US 6,197,352 discloses the addition of protease for weakening gluten, in particular when using hard wheat flour and US 4,851,234 discloses a process for preparing an anti-staling agent for baked goods in which protease is used.

11. Regarding the ratio of activities at 25C and the optimum temperature for protease activity, it is obvious that a thermostable enzyme with a high optimum temperature for activity will be much more active at high temperatures (high optimum temperature) than the same enzyme at much lower temperature e.g. 25C. The ratio of activity at a much higher temperature (thermostable enzyme) to the activity of the same enzyme at lower temperature, e.g. 25C, will be intrinsically high as presently claimed.

12. For examination purposes the phrase "has no perceivable action on the dough rheology" is assumed to mean that no change in consistency of the dough takes place. However this behavior by the dough is expected when a thermophilic protease is added to the dough. Thermophilic enzymes are apparently inactive at ambient temperatures therefore due to extremely low reaction rates, it appears that the consistency does not change.

13. R1 is silent regarding addition of other enzymes and emulsifier to the dough.

14. R2 discloses the use of lipase together with other enzymes such as cellulase, hemicellulase, xylanase, glucose oxidase, peroxidase, amyloglucosidase, and alpha-amylase in bakery products including bread (Col. 5, lines 33-46). Bacterial alpha-amylase is known in the art and is a thermostable enzyme. As stated in paragraph 9 above, the use of amylase and protease to retard staling in bread was known in the art at the time the invention was made, therefore; it would be obvious to those of skill in the art to select a thermostable amylase such as a bacterial amylase to add to the dough formulations so that the thermostable amylase and thermostable protease are activated at higher temperatures usually encountered at the beginning of the baking in the oven.

15. R2 discloses that examples of other enzymes are a cellulase, a hemicellulase, a pentosanase such as xylanase (useful for the partial hydrolysis of pentosans which increases the extensibility of the dough), a glucose oxidase (useful for strengthening the dough), e.g. a fungal glucose oxidase such as Novozym 358.RTM. (a A. niger glucose oxidase), a protease (useful for gluten weakening in particular when using hard wheat flour), e.g. Neutrase.RTM., a peroxidase (useful for improving dough consistency), a peptidase, a maltogenase, and/or an amylase, such as an amyloglucosidase (e.g. AMG.RTM. (an A. niger amyloglucosidase) and an .alpha.-amylase (useful for providing sugars fermentable by yeast). The other enzymes are preferably of microbial origin and may be obtained by conventional techniques used in the art as mentioned above. (col. 5, lines 33-47).

16. R2 teaches using emulsifiers such as mono and diglycerides, diacetyl tartaric acid esters of mono- and diglycerides (DATEM), sugar esters of fatty acids, lactic acid esters of monoglycerides, polyoxyethylene stearates, phospholipids and lecithin in their dough improver (Col. 6, lines 46-56). These emulsifiers are used to improve dough extensibility as well as the consistency and storage stability of the bread; therefore they help retard staling of baked goods.

17. It would have been obvious to one of ordinary skill in the art, at the time the invention was made, to use a thermostable protease as taught by R1 and include the improving enzymes and emulsifiers as taught by R2 for the benefits of the dough improving properties of such enzymes and emulsifiers to prevent or retard staling in baked goods. Since the combination of protease and other anit-staling enzymes and emulsifiers were known in the art to retard or prevent staling in baked goods, the combination of protease, amylase and emulsifiers presently claimed are expected to retard or prevent staling in baked goods. Absent any evidence to contrary and based on the combined teachings of the cited references, there would have been a reasonable expectation of success in retarding or preventing staling in baked products.

18. Claims 7 and 16 are rejected under 35 U.S.C. 103(a) as being unpatentable over R1 in view of Terada et al. (US 5,124,261; hereinafter R3) and Chernoglazov et al. (RU 2,177,799; hereinafter R4).

19. R1 teaches preparing a heat stable thermitase from *Thermoactinomyces vulgaris* which is used in baking process as described above. R1 is silent regarding protease of *Thermus aquaticus* and Keratinase of *Bacillus licheniformis.*

20. R3 discloses a process for the production of aqualysin I employing a genetic engineering procedure by cultivation of *Thermus aquaticus* (Col. 1, lines 34-52 and Col. 8, lines 31-51).

21. R3 is silent regarding a keratinase enzyme.

22. R4 discloses a new keratinase from *Bacillus licheniformis*. The keratinase can be used in the food industry (Abstract).

23. It would have been obvious to one of ordinary skill in the art, at the time the invention was made, to modify the teachings of R1 by including the protease and keratinase taught by R3 and R4. One would do so for the benefits of a thermostable protease and keratinase at least at the early stages of baking where the temperature is high enough for the activation of these thermostable enzymes and yet not that high to denature such enzymes. Since the combination of protease, amylase and emulsifiers were known in the art to retard or prevent staling, the combination of enzymes and emulsifiers as presently claimed would be expected to retard or prevent staling in baked goods. It is clear that the weakening of gluten takes place at a higher temperature at the beginning of baking in the oven. Absent any evidence to contrary and based on the combined teachings of the cited references, there would have been a reasonable expectation of success.

24. Claims 5, 23, 24, 26, and 28-29 rejected under 35 U.S.C. 103(a) as being unpatentable over R1 as applied above, further in view of Stetter (US 5,714,373; hereinafter R5).

25. R1 teaches of preparing a heat stable thermitase from *Thermoactinomyces vulgaris* and using it in baked products as described above.

1. R5 discloses the isolation and identification of a thermostable protease from *Thermococcus* which has an optimum temperature range between 60C and 90C (col.7, lines 38-41).

2. It would have been obvious to one of ordinary skill in the art to use proteases which have an optimum range of activity in the 60C-90C as disclosed by R5. It is clear that the higher the optimum temperature of the protease used in the dough, the higher the temperature of baking will be tolerated at the beginning of the baking in oven.

3. Claims 5 and 26 are obvious due to the fact that serine proteases have a serine residue at the active site which acts as a nucleophilic residue in proteolytic activities, being active at neutral or alkaline pH. Aqualysin I and aqualysin II were known in the art as alkaline and neutral proteases respectively; at the time the invention was made.

4. It would have been obvious to one of ordinary skill in the art, at the time the invention was made, to employ the thermostable proteases from various sources as taught by R1, R3, R4 and use them optimally at 60-90C as taught by R5. The higher optimum temperature of these thermostable proteases will help the weakening of gluten in the dough at higher temperatures encountered at the beginning of baking in the oven before they are denatured at higher oven temperatures later during the course of baking.

5. Claim 34 is rejected under 35 U.S.C. 103(a) as being unpatentable over R1 as applied above, further in view of Matsuzawa et al. (1988, Purification and characterization of aqualysin I.; hereinafter R6).

6. R1 discloses the application of a thermostable protease in baking process as outlined above. R1 is silent regarding the used of aqualysin II.

7. R6 discloses that *Thermus aquaticus* produces both aqualysin I and II. Aqualysin I is an alkaline protease and aqualysin II is a neutral protease. Optimum temperatures for proteolytic activities of the two enzymes being around 75C and 95C respectively. (page 441, col. 1, third paragraph).

8. Therefore, it would have been obvious to one of ordinary skill in the art to modify the teachings of R1 and substitute the thermitase of R1 with aqualysin II as taught by R6. One would do so to benefit from an enzyme with a higher optimum temperature. It is clear that such an enzyme would endure higher temperatures encountered in the oven at the beginning of the baking process. Absent any evidence to contrary and based on the combined teachings of the cited references, there would be a reasonable expectation of success in using a thermostable proteases with a higher optimum temperature for activity.

Response to Arguments

Applicants' arguments have been thoroughly reviewed. They are not deemed persuasive for the following reasons.

1. Applicants argue that Neither R1 nor R2 teaches or suggests the following features in present claim 1.

- the antistaling effect of thermostable serine protease and the addition of an amount of protesase which prevents or retards staling of a bakery product.
- the optimum temperature of the thermostable serine protease higher than 60C
- the ratio of activity at optimum temperature and the activity at 25C higher then than 10.
- lack of perceivable action of protease on dough rheology before the baking process.

a. All of the argued features are either expressly or implicitly disclosed by R1. R1 uses a thermitase of thermoactinomycess vulgaris as presently claimed. The optimum temperature of this protease is 60-70C as disclosed by R1. R1 discloses the weakening of gluten in the dough and since weakening of gluten in the dough was known, as one of the factors, to affect the retardation of staling in baked product, then anti-staling effect of this thermostable protease is implied by R1. The optimum temperature for this enzyme was known as early as 1969. Please see Odibo, F.J.C. et al. 1988. MIRCN Journal. 4: 327-332. This enzyme has an optimum temperature for activity at 70C. Regarding the ratio of activities at 25 C and optimum temperature of the thermophilic enzyme, it is believed that this property is intrinsic in all thermophilic enzymes. The higher the optimum temperature, the higher would be this ratio. The reason is that at ambient temperature , e.g. 25 C, a true thermophilic enzyme is basically apparently inactive regarding the hydrolytic activity. As the temperature of the reaction is increased,

hydrolysis starts and increases as the temperature approaches the optimum. It is very obvious that at optimum temperature a thermophilic enzyme would be at least 10 times as active as it is at 25 C.

2. Applicants argue that the purity of thermostable enzyme causes the ratio of activity at optimum temperature to be at least 10 times as much as the activity at 25C.

a. The examiner does not agree with this statement. The purity of an enzyme will affect the specific activity of the enzyme i.e. activity per unit mass of the enzyme protein. A higher ratio of activity at an optimum high temperature compared to the activity at ambient is expected for a thermophilic enzyme. In other words, when a partially pure thermophilic enzyme is assayed at ambient temperature and at optimum temperature, the ratio of the activities at these temperatures will be the same as the ratio of activities when a pure enzyme is assayed at ambient temperature and optimum temperature. This is an inherent property of the enzyme and has nothing to do with the purity of enzyme. However, it is assumed that the impure enzyme preparation does not contain inhibitors or denaturants which generally affect the activity at any given temperature.

3. Applicants argue that the serine protease having the recited properties can prevent or retard staling while having no perceivable effect on dough rheology. They argue that there is no teaching or suggestion of using the serine protease in a sufficient amount to achieve these results.

a. Inclusion of proteases in bread dough as an anti-staling agent has been known in the art for many years. Please see paragraph 10 above for patents addressing anti-

staling effects of proteases. The following passage is from Gray, J. A. et al. 2003 (Bread staling, Molecular basis and control. Comprehensive Reviews in Food Science and Food Safety. 2: 1-21. At bottom of page 10 " Van Eijk and Hille (1996) concluded that while the addition of excess concentrations of proteolytic enzymes would certainly be detrimental to the bread loaf, adding optimal levels of proteases to breads might increase their shelf life."

It is noted that since other factors are result effective in retarding the staling in baked products, it is the Examiner's belief that the word "might", in the above passage, acknowledges that proteases and breakdown of gluten are not the only effective variables in the process of the prevention of staling.

Then it is clear that proteases at optimal concentrations are beneficial processing aids in increasing the shelf life of bread; i.e. they act as anti-staling agents.

Further, R1 discloses the weakening of gluten, therefore, the serine protease of R1 is being used at enough concentration to affect the weakening of gluten and consequently the anti-staling effect, partially due to the effect of the protease, is expected.

4. Applicants argue that they do not concede that the crude enzyme preparations discussed in Klingenberg necessarily disclose serine proteases having the properties recited in the claims.

a. Klingenberg (R1) utilizes thermitase, the thermophilic protease from thermoactinomyces vulgaris having an optimum temperature for activity at 60-70C. This enzyme has been presently claimed. Therefore, the effects observed in R1 will be the

same as far as the gluten is concerned. Applicants are utilizing the same enzyme as disclosed by Klingenberg, therefore, it would be obvious to expect the same results.

5. Applicants argue that Klingenberg does not describe any partially or highly purified forms of thermitase.

a. The paragraph quoted by Applicants mentions crude preparation, partially purified form and in highly purified form as disclosed by Klingenberg.

Even if such a disclosure was not provided by Klingenberg, those of skill in the art know that enzymes are prepared (from biological sources) in crude form, they are then partially purified (at certain steps of purification) and in order to be studied or used for specific purposes, they are highly purified (high specific activity). The determination of the form of the enzyme for a specific application is within the skill of the art. When a less pure enzyme is used, more of the enzyme preparation is needed for obtaining the desired effect. R1 discloses the weakening of gluten through using the thermostable protease, therefore, it is understood that enough enzyme has been used by R1 for the desired effect being weakening of gluten.

6. Applicants argue that not all thermostable proteases are suitable for anti-staling effect and as an example they assert that papain would not be suitable.

a. Papain is not disclosed by any of the references used in rejections. Papain is not claimed by applicants. Therefore, it would be irrelevant to discuss the properties of papain. R1 is using the thermophilic protease, thermitase, in baking process which is currently being claimed. Therefore, it would be obvious to use it in baking.

7. Applicants argue that it is interesting to note that after the present invention was made, the staling phenomenon had still not been elucidated.

a. Please see paragraph 10 above for patents dealing with the staling phenomenon. Furthermore, the staling phenomenon is a complex phenomenon which has been the focus of research for years. Therefore, per prior art, addition of protease to the dough may be just a part of solving the puzzle.

8. Applicants argue that R1 neither discloses nor suggests using thermitase in a baking process.

a. This statement is a mischaracterization of R1. R1 teaches of using thermitase in baking.

9. Applicants argue that the examiner has taken a passage out of context in order to support his allegation that proteases extend the shelf life of bakery product.

a. The examiner does not agree with this statement. The reference indicates that the role of protease to remedy, at least partially, the staling problem in baking products was known in the art at the time the invention was made.

9. Applicants argue that although R3 discloses aqualysin 1, this reference neither teaches or suggests that this enzyme can be used in baking processes or more generally in the food industry.

a. The primary reference R1, discloses the use of thermostable protease in baking process. R3 is not required to repeat the same concept. R3 is a teaching reference indicating that aqualysin I was known in the art as a thermophilic protease at the time

the invention was made. Therefore, using it in baking would be obvious per the disclosure by R1.

5. Applicants argue that it is unknown what the effects of adding the crude preparations described in R1 to the dough would be. In particular it is not known whether such crude preparations could prevent or retard staling while having substantially no effect on dough rheology.

a. As mentioned earlier, the contribution of proteases to retarding or prevention of staling in bread was known before the invention was made. Even if a partially purified enzyme was used by R1 (the examiner is not aware of the purity of enzyme due to the language of the publication), what needed to be done by applicants was to determine the activity and the concentration of the enzyme to be used in preparing the dough which were both within the skill of the art. A skilled artisan knows that when the crude preparation is too dilute for the desired effect, the preparation should be concentrated or partially purified or even highly purified by utilizing conventional techniques in the art. It should be realized once the use of thermitase in baking was disclosed by R1, manipulations of enzyme forms, the concentration used, and optimization of the levels for a desired effect would all be within the skill of the art.

Conclusion

26. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. US 6,197,352. This patent discloses the anti-staling compositions for bread comprising amylase and protease. The following patents are also referred to

but not relied upon in the current Office action. US 6,270,813; US 6,197,352; and US 4,851,234.

27. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL.** See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to HAMID R. BADR whose telephone number is (571)270-3455. The examiner can normally be reached on M-F, 8:00-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Keith Hendricks can be reached on (571) 272-1401. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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